AFRRI SCIENTIFIC REPORT

AFRRI SR73-2
FEBRUARY 1973

EFFECT OF A SUPRALETHAL DOSE OF RADIATION ON THE BLOOD-BRAIN BARRIER

A. A. René
J. L. Parker
J. H. Darden
N. A. Eaton

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.

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A. A. RENE
J. L. PARKER
J. H. DARDEN
N. A. EATON

S. J. Baum
S. J. BAUM
Chairman
Experimental Pathology Department

Myron I. Varon
MYRON I. VARON
Captain MC USN
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of M. L. Guimond.

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FOREWORD
(Nontechnical summary)

Under normal conditions the central nervous system has a unique ability for restricting the passage of various substances from the blood vessels into the surrounding brain tissue. This special property exists only in brain tissue and has been attributed to the presence of a barrier between the blood and the brain tissue, suitably termed the blood-brain barrier.

This barrier may be changed by head injuries or other adverse conditions, such as radiation, increasing its permeability and permitting the accumulation of fluid in the brain tissue. The presence of an abnormal amount of fluid in the brain can cause a central nervous system malfunction. Of considerable interest is the mechanism by which this fluid is transported.

The use of a tracer has been employed in the present study to determine how this fluid transfer may develop. Horseradish peroxidase (HRP), an enzyme, was intravenously injected into irradiated and control nonirradiated animals. The medullary sections of the brain of the injected animals were removed after they were fixed by immersion. The sections were cut into 50 μm slices and placed in a special incubation medium containing a substrate to the enzyme. The enzyme and the substrate form a reaction product at the site of action (in the vicinity of the blood-brain barrier) which can be viewed as an electron opaque material under the electron microscope.

The examination of electron micrographs of the brain tissue of irradiated and nonirradiated control animals indicates that the mode of transcapillary passage of HRP is apparently micropinocytosis (transport across the cells lining blood vessels)

and not simple diffusion through the blood vessels or through junctional gaps between the endothelial cells which form the wall of the blood vessels. It appears, then, that damage caused by high doses of radiation initiates increased vascular permeability which may lead to the edematous condition observed in the irradiated animal.

ABSTRACT

Sprague-Dawley rats were subjected to a whole-body dose of 15 krads of mixed gamma-neutron radiation. Following irradiation, the animals were intravenously injected with horseradish peroxidase (HRP) and sacrificed. The medullae of these animals were fixed either by perfusion or by immersion, incubated in a substrate medium and processed for electron microscopic examination. In control animals subjected to the same procedures, the reaction product of the enzyme HRP and its substrate was confined to the lumina of the blood vessels in the brain fixed by immersion and completely washed out of the blood vessels of the brain fixed by perfusion. The brain tissue of the irradiated animals injected with HRP and fixed by immersion exhibited a dense reaction product in the lumina of the blood vessels, in invaginations of the luminal surface, and in micropinocytotic vesicles of the endothelial cell cytoplasm. The electron dense material was also observed in the outer margin of the basement membrane and in areas beyond this membrane. The brain tissue of the irradiated animals fixed by perfusion was similar to the tissue fixed by immersion except for the presence of reaction products within blood vessels. The most significant alteration in the medullary tissue of the irradiated animals was an increase in the number of micropinocytotic vesicles in the endothelial cells. This was viewed as the mode of transcapillary passage of substances from the vascular tissue to the parenchymal tissue of the brain. It appears, then, that damage caused by high doses of radiation initiates increased vascular permeability which may lead to the edematous condition observed in the irradiated animal.

I. INTRODUCTION

The existence of a blood-brain barrier (BBB) in the central nervous system has been well established. Under experimental conditions, alterations in the BBB have been induced by various means, including radiation,[4,6] resulting in increased passage of dyes and vascular fluid into the brain tissue. Although a number of papers describe the radiation-induced BBB changes,[6,12,17] their relationship to the formation of edema has not been established.

The objective of this study was to determine how edema may possibly develop in the brain tissue of animals exposed to relatively high doses of radiation. Electron microscopy was employed to study a low molecular weight protein, horseradish peroxidase (HRP), as a tracer suitable for locating permeability changes in the BBB. Increased permeability may be the cause of possible fluid loss from blood vessels and edema of certain tissue.

II. MATERIALS AND METHODS

One hundred and sixty Sprague-Dawley rats of the Charles River strain, weighing approximately 200 grams, were used. These animals were divided into two groups. One group was exposed to a pulse of 15 krads of whole-body mixed gamma-neutron radiation from the AFRRI-TRIGA reactor while the other served as nonirradiated controls. These two groups of animals were subsequently further divided into two groups, one injected intravenously with 33 mg of horseradish peroxidase dissolved in 1.0 ml of isotonic saline while the other group received only saline. The peroxidase used was of two types, Sigma type II (RZ value 1.0 - 1.5, Sigma Chemical Co., St. Louis, Missouri) and electrophoretically purified peroxidase (RZ value 2.9 - 3.0, Worthington

Biochemical Corporation, Freehold, New Jersey). The peroxidase was injected 5 - 30 minutes before the animals were sacrificed. At sacrifice, the animals were anesthetized with an intraperitoneal injection of 50 mg of Nembutal (sodium pentobarbital, Abbott Laboratories) per kg body weight and the brain fixed by perfusion[7,9,18,24] or by immersion. Although perfusion fixation was far superior to immersion fixation in terms of the natural appearance of the tissue, the presence of the tracer in the endothelial cell lumina provided for a more positive identification of the peroxidase activity.[20] Immersion fixation required that brain tissue be rapidly removed and placed in cold (4^{o}C) 3 percent glutaraldehyde buffered with 0.1 M cacodylic acid.

Prior to the perfusion procedure, the trachea was cannulated with a polyethylene tube (i.d. .062", o.d. .082") which provided a continuous low-level flow of oxygen from a bottle of compressed oxygen containing 5 percent CO_2. The connecting tube was designed to permit the intermittent inflation of the lungs simulating normal respiration while the thoracic cavity remained open. A polyethylene intravenous tubing (i.d. .045", o.d. .062"), connected to the perfusate reservoir, was tied into the descending aorta. The perfusate was 3 percent glutaraldehyde buffered with 0.1 M cacodylic acid, maintained at 4^{o}C. A small amount of saline with 2 percent heparin was used as a preperfusate and placed into a length of the perfusion tube. Before the perfusion procedure was allowed to begin, the ascending aorta was sutured and the right atrium severed for circulatory drainage. The flow of the perfusate was started immediately at 120 mm Hg and then lowered to 100 mm Hg after most of the blood had been flushed out of the animal (indicated by a yellowing of the nose and ears). The pressure was determined by the height of the liquid in the perfusate reservoir. The

perfusion of the animal was continued for 20 minutes, after which the rat brain was removed, the medulla oblongata dissected and cross sections collected in the area caudal to the midpoint of the pons and cranial to the obex or upper limit of the spinal cord. Sections from both immersion-fixed and perfused brains were cut into slices 50 μm in thickness in a Smith-Farquhar tissue chopper[25] and incubated in a special substrate medium for 15 minutes. This substrate consisted of 5 mg of 3',3'-diamino-benzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, Missouri) dissolved in 10 ml of 0.05 M Tris buffer, pH 7.6. One milliliter of 1 percent H_2O_2 (freshly prepared from 30 percent H_2O_2) was added to the medium to give a final concentration of 0.01 percent H_2O_2.[10] The sections were then washed overnight at 4^oC in 0.1 M cacodylate buffer containing 3 percent sucrose and postfixed in 1 percent osmium tetroxide buffered to pH 7.4 with s-collidine.[2] The sections were washed in cold 0.05 M sodium hydrogen maleate (known as NaOH buffer) buffered at pH 5.2 and then en bloc stained by covering the tissue with 2 percent uranyl acetate dissolved in 0.05 M buffer, pH 6.0. The tissue was allowed to stain for 2 hours in complete darkness at 4^oC, washed in three changes of NaOH buffer pH 5.2 and dehydrated and embedded in Epon.[14] The blocks were cut with a Porter-Blum MT2 ultramicrotome and sections mounted on uncoated grids. After staining with lead citrate[23] the sections were examined in a Siemens electron microscope. The reaction product of the horseradish peroxidase and its substrate appeared in the circulation or tissue as an electron opaque material.

III. RESULTS

The ultrastructure of the brain tissue of normal nonirradiated animals injected with the tracer did not differ from that of animals not injected. These results did not

change significantly even when the peroxidase remained in the bloodstream for 1 hour before the animals were sacrificed.

The peroxidase activity was confined to the lumina of the capillaries of control animals whose tissue had been fixed by immersion (Figure 1).[20] The radiation dose used did not cause a drastic change in fine structure within 2 hours after exposure. There were, however, some subtle changes which were clearly significant. The brain tissue of the irradiated animals showed an increased number of pinocytotic vesicles within the cytoplasm of the vascular endothelial cells. The examination of the brain

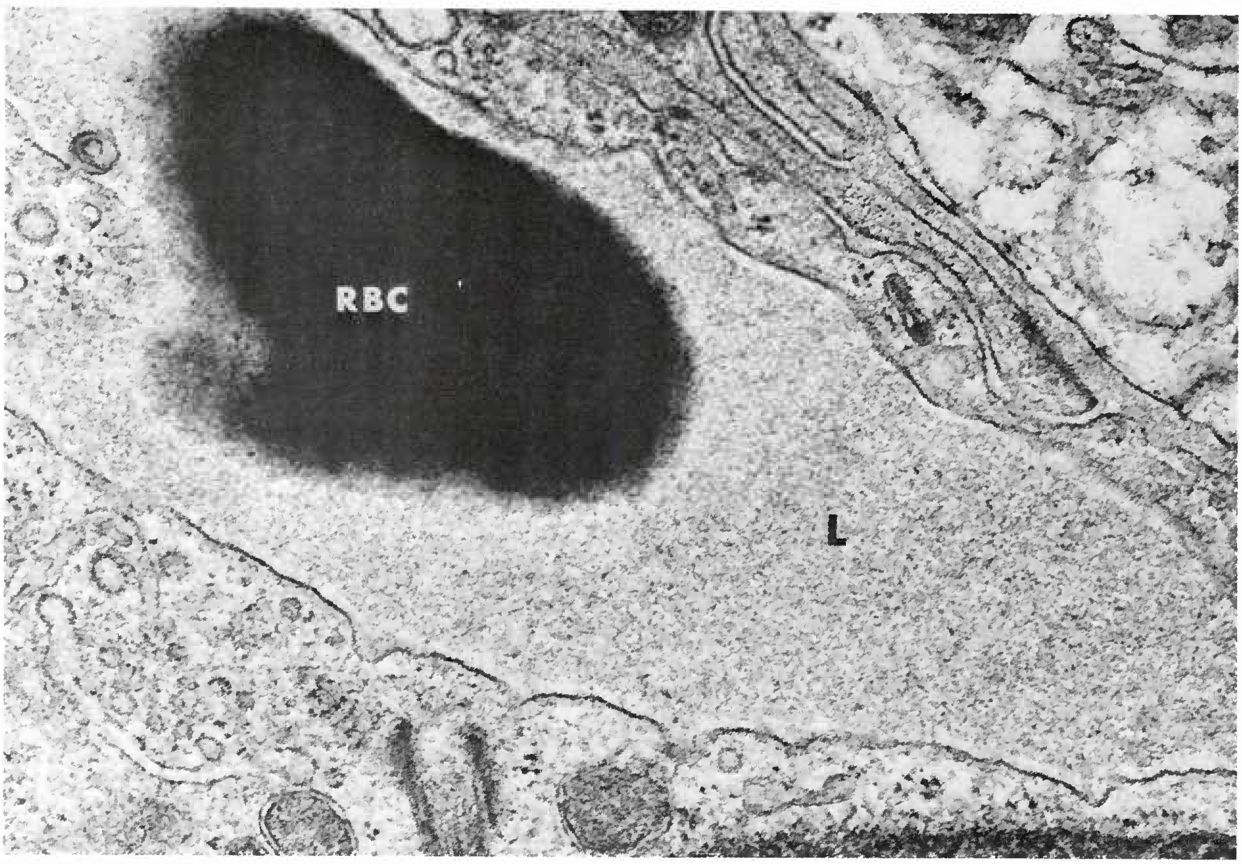

Figure 1. A cross section of a medullary capillary of a control rat. The reaction product and a red blood cell may be seen in the lumen (L) of the vessel. A red blood cell (RBC) may also be seen in the capillary lumen. X 68,875

tissue of exposed animals injected with the tracer and fixed by immersion revealed peroxidase activity in the lumina of the blood vessels, in deep invagination of the endothelial luminal surface, and within numerous micropinocytotic vesicles in the endothelial cell (Figures 2 and 3). The peroxidase activity was occasionally found within the endothelial cell junctions and in micropinocytotic vesicles at the contra-luminal margin of the basement lamina (Figure 3) and in areas beyond the basement lamina (Figure 4).

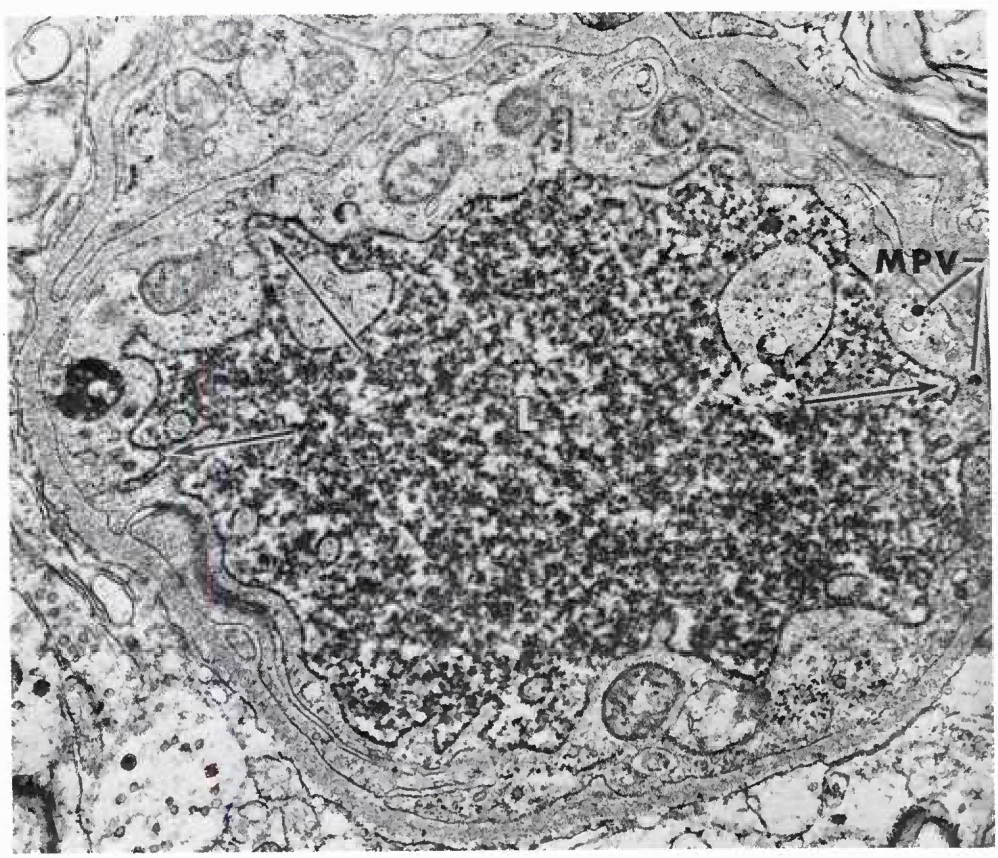

Figure 2. A cross section of a medullary capillary of a rat exposed to 15 krads of whole-body mixed gamma-neutron radiation. The reaction product may be seen in micropinocytotic vesicles (MPV) and invaginations (arrows) of the endothelial cells in addition to the lumen (L) of the blood vessel. X 21,875

Peroxidase activity was not observed to any appreciable amount in the extracellular spaces of brain tissue. These results did not change significantly when the peroxidase was allowed to remain in the bloodstream of the animals for up to 1 hour before sacrifice.

The neurons of the irradiated specimens showed an increased number of lipofuscin granules and lysosomes (Figure 5a, b). The Golgi apparatus appeared to be larger and in greater numbers in these cells. The mitochondria exhibited a slight

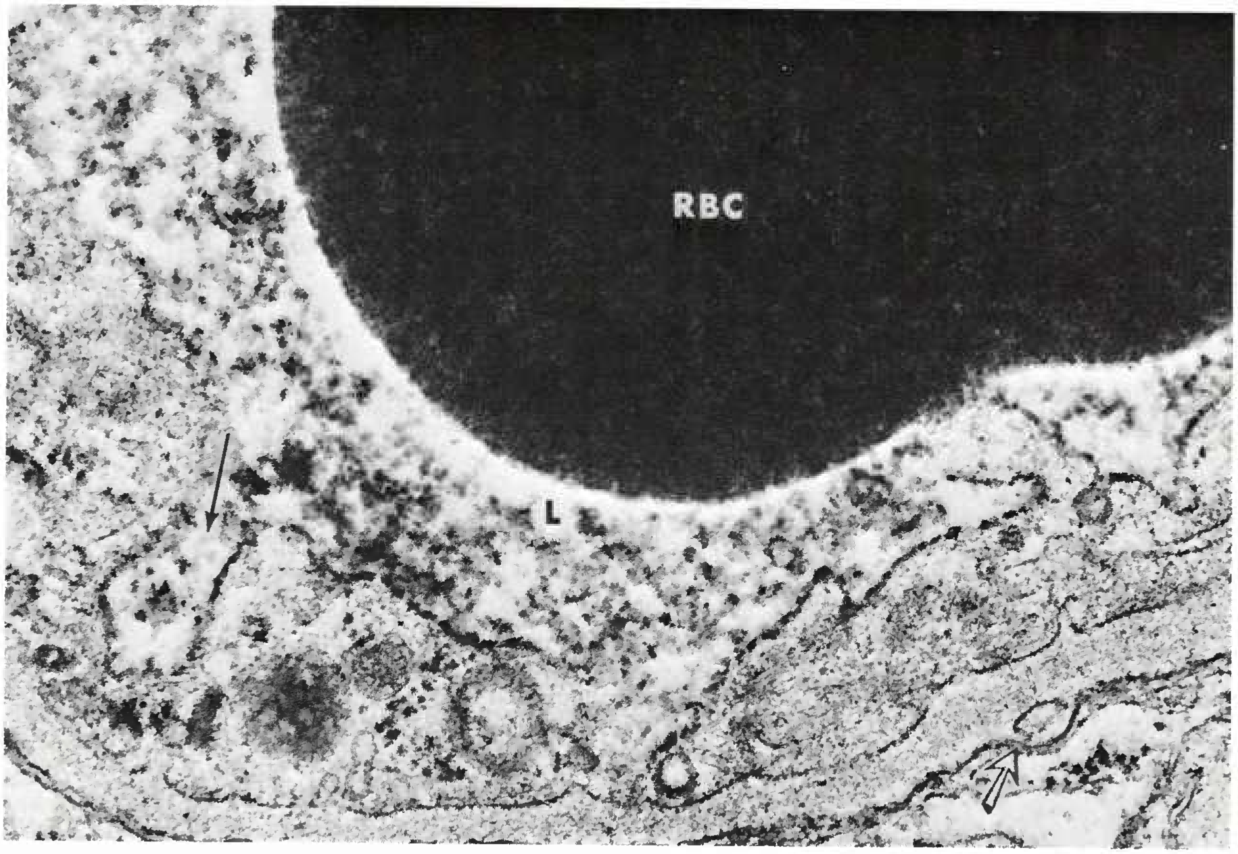

Figure 3. A cross section of a medullary capillary of a rat exposed to 15 krads of whole-body mixed gamma-neutron radiation. The reaction product may be seen in invaginations of the endothelial cell (solid arrow), in the lumen (L) of the vessel and in a vesicle on the contraluminal margin of the basement lamina (open arrow). A red blood cell (RBC) may also be seen in the capillary lumen. X 53,750

inflation of the cristae with only an insignificant dilution of its matrix. Most glial cells of the irradiated animals showed observable differences in the morphology of the nuclear envelope and the rough endoplasmic reticulum (Figure 6a, b). An increase in glycogen granules of these cells was not observed.

The nerve and glial cell processes of the irradiated animals contained their usual complement of subcellular organelles with little observable differences, when compared to the cell processes of nonirradiated animals, aside from lipofuscin granules and Golgi apparatus.

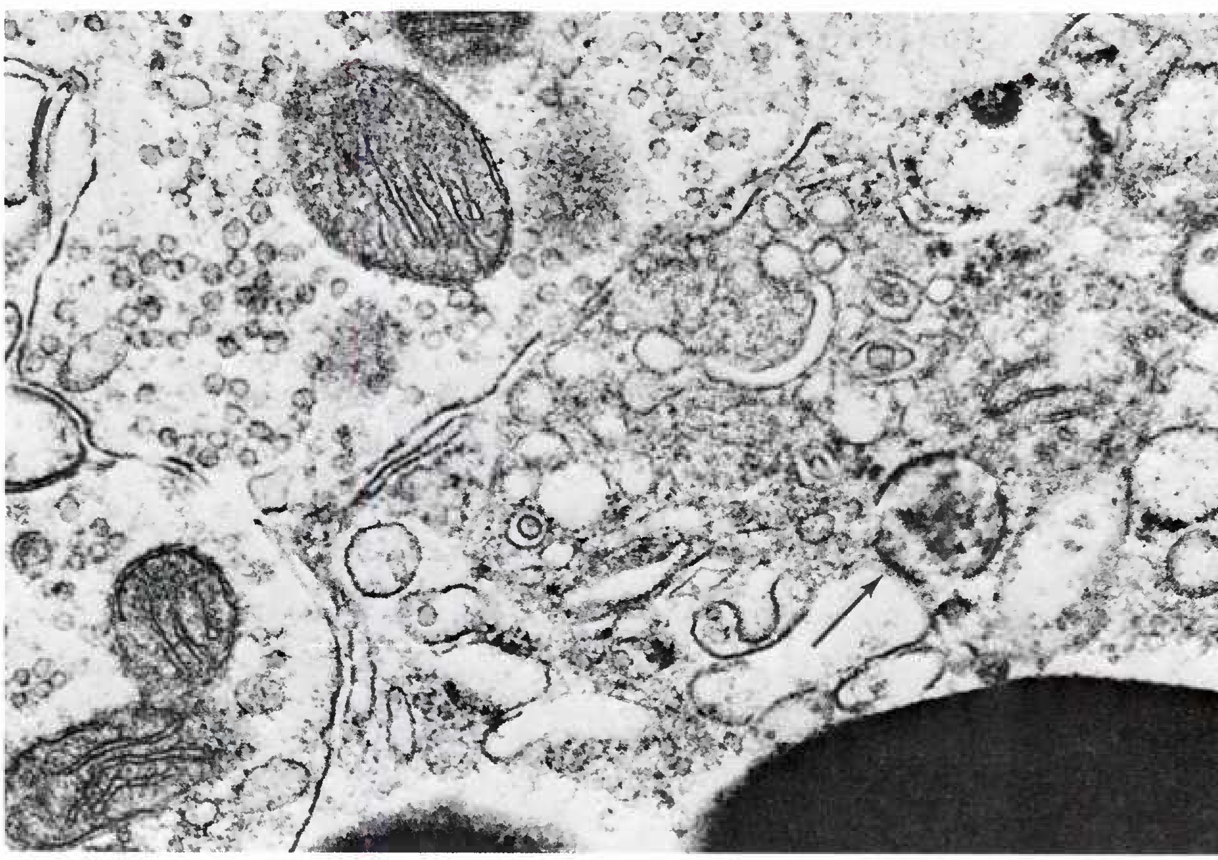

Figure 4. A section of medullary parenchymal tissuc of a rat exposed to 15 krads of whole-body mixed gamma-neutron radiation. Reaction product in a vesicle may be seen in what appears to be glial cell cytoplasm (arrow). X 50,000

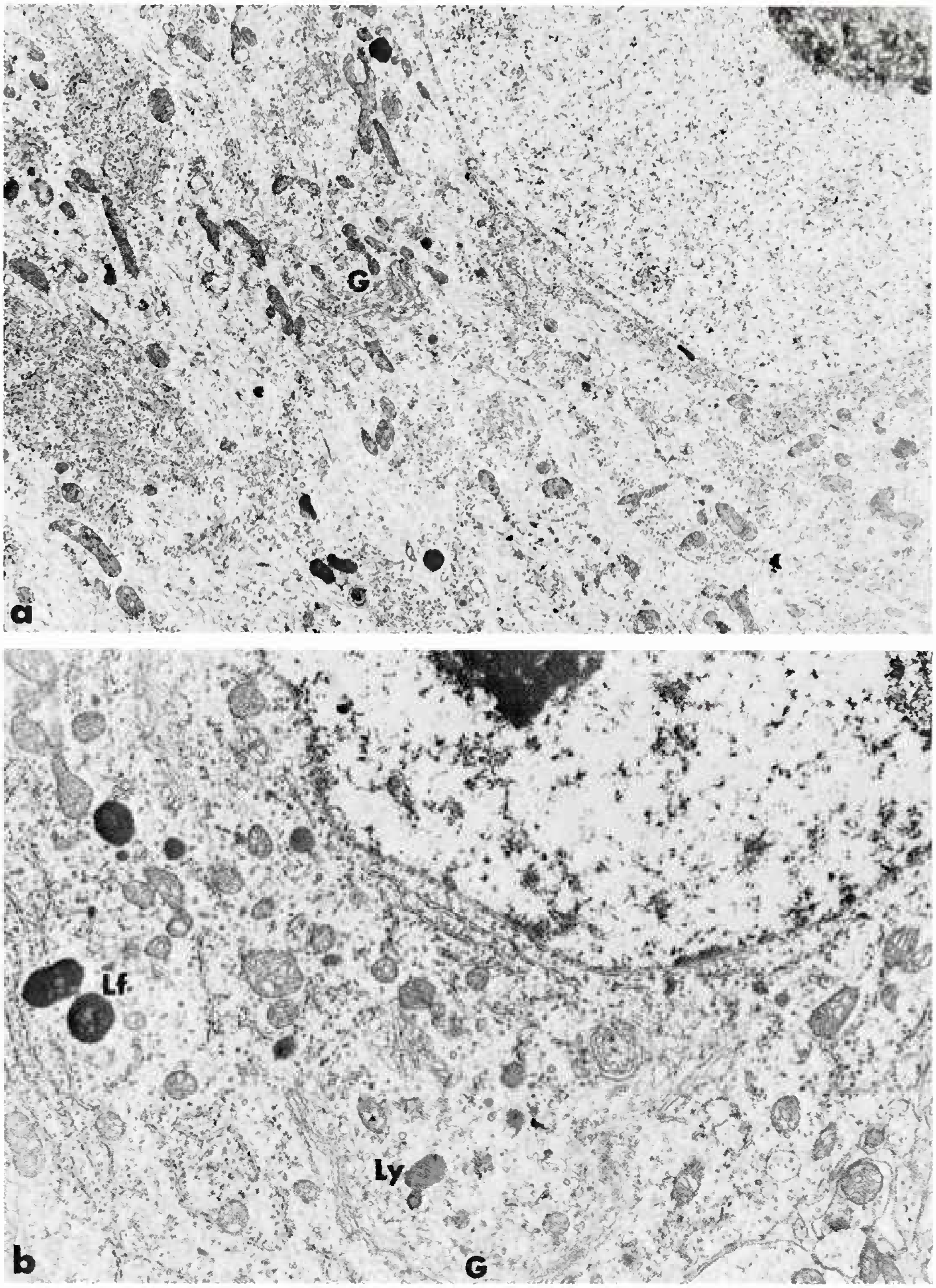

Figure 5. Medullary neurons of a normal rat (a) and a rat exposed to 15 krads of whole-body mixed gamma-neutron radiation (b). Lipofuscin granules (Lf) and lysosomes (Ly) may be seen along with the Golgi apparatus (G) and other cytoplasmic organelles. X 10, 300

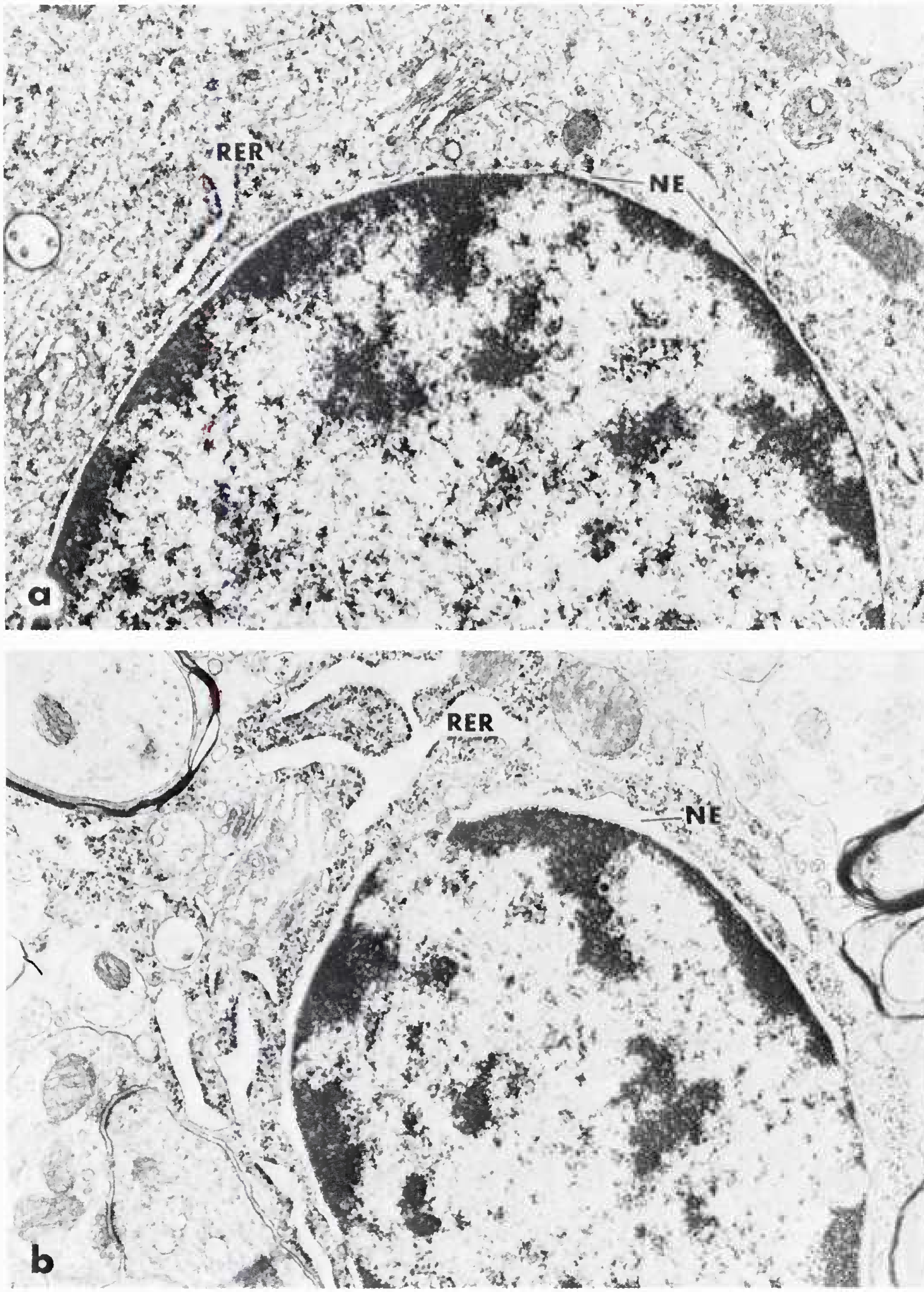

Figure 6. Medullary oligodendrocytes of a normal rat (a) and a rat exposed to 15 krads of whole-body mixed gamma-neutron radiation (b). Enlarged rough endoplasmic reticulum (RER) and nuclear envelope (NE) may be seen. X 20,595

IV. DISCUSSION

It has been established not only that a permeability change in the capillaries of cardiac and skeletal muscle involves an alteration in the endothelium but that peroxidase crossed the capillary endothelium by two means, i.e., pinocytotic vesicles and junctional channels between endothelial cells.[10] Therefore, the failure of circulating peroxidase to cross the endothelium of brain capillaries might well be due to the absence of these channels and the low rate of transport in pinocytotic vesicles.[20]

Since it is generally believed that the separation of the endothelial cells (junctions) constitutes an important factor in the formation of protein-rich inflammatory edema in tissues outside the nervous system,[15] one would expect this to be true for brain tissues. However, the present study has indicated that relatively high doses of radiation increase pinocytotic activity with no apparent change in the endothelial cell junctions.

These observations do not contradict the idea that, of the two means of transcapillary transport, the endothelial tight junction constitutes the main morphological substrate of the BBB for protein.[3] They merely support the suggestion that under certain pathological conditions an intensified pinocytotic activity could ferry appreciable amounts of protein across the endothelium.[5]

Since there are no observable postirradiation changes in the endothelial cell junctions, yet there is a definite noticeable increase in micropinocytotic vesicle activity, it is quite logical to assume that the latter may become the dominant means of transcapillary passage in edema formation. According to our observations, this is apparently true. It is further substantiated by a number of reports[1,5,8] which state

that phagocytosis and pinocytosis of a significant amount of colloidal tracer or fluid materials from the capillary lumen probably occur only under abnormal conditions. However, this assumption appears to be contrary to a previous suggestion that pinocytosis should be more efficient under optimal physiological conditions than under pathological conditions and that pinocytosis is normally a comparatively slow process.[3]

The tracer used in the present study has implicated pinocytosis as the possible cause for increased permeability and subsequent edema in irradiated animals initiated by transcapillary passage of particles comparable in size to that of a molecule of HRP. Smaller molecules which contribute to edema formation may also utilize the tight junctions or diffusion directly across endothelial cells as a passageway.

Some of the radiation-induced changes in other areas of the central nervous system (CNS) may also indicate a cellular edematous condition. These pathologic conditions have been observed in other organs. For example, the increase in Golgi activity of irradiated mouse hepatocytes was not unlike that of the neurons of the irradiated animals of the present study. René and Evans[22] interpreted this condition as a compensatory attempt of the hepatocytes to eliminate excessive amounts of products accumulated in the tissue of exposed animals. The number and complexity of the Golgi apparatus vary directly with the secretory activity of various types and different functional states of cells.

The changes in the nuclear envelope and the rough endoplasmic reticulum (RER) which were observed in the glial cells of the CNS tissue of irradiated animals may reflect an increase in cellular water.[26] This postirradiation condition of the RER has also been demonstrated in mouse hepatocytes.[22]

The third subcellular element which reflected the presence of an increased amount of cellular fluids is the slight swelling in the mitochondria. This condition could, however, be associated with suboptimal fixation, an anoxic environment or a depressed rate of phosphorylation.[26] Although there is a wide variability of maximum swelling amplitude among mitochondria, brain mitochondria cannot swell more than 1 - 2 percent of their volume.[13] This observation seems to indicate that although the mitochondrial swelling in the present study appeared to be minor, what has been observed in the neurons may be of major proportion and could be related to the overall necrobiotic effect of radiation. The increase in the number of lysosomes in the brain tissue of irradiated animals is indeed related to cellular necrobiosis following irradiation.[21] The major constituents of the lipofuscin granules produced by a partial degradation of unsaturated lipids are believed to be residual bodies derived from lysosomes.[19] Although a striking accumulation of glycogen granules has been reported in astrocytes of animals exposed to radiation,[11] it was not observed in the present study. The accumulation of glycogen occurring immediately after irradiation is very likely a direct effect of radiation upon the metabolic machinery of the astrocyte since it occurs before any sign of tissue injury.[16]

The edematous condition which develops in brain tissue of animals exposed to relatively high doses of ionizing radiation is due to an increased vascular permeability. According to the present study, this pathologic condition is evidently not due to a change in the endothelial cell tight junctions but is apparently the result of an enhanced micropinocytosis capable of increasing transcapillary passage of protein-rich vascular fluid into the brain parenchymal tissue. It appears, then, that damage caused by high

doses of radiation initiates increased vascular permeability which may lead to the edematous condition observed in the irradiated animal.

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UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)* Armed Forces Radiobiology Research Institute Defense Nuclear Agency Bethesda, Maryland 20014	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP N/A

3. REPORT TITLE

EFFECT OF A SUPRALETHAL DOSE OF RADIATION ON THE BLOOD-BRAIN BARRIER

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(First name, middle initial, last name)*

A. A. René, J. L. Parker, J. H. Darden and N. A. Eaton

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
February 1973	21	26

8a. CONTRACT OR GRANT NO. b. PROJECT NO. NWED QAXM c. Task and Subtask C 902 d. Work Unit 03	9a. ORIGINATOR'S REPORT NUMBER(S) AFRRI SR73-2 9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*

10. DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY Director Defense Nuclear Agency Washington, D. C. 20305

13. ABSTRACT

Sprague-Dawley rats were subjected to a whole-body dose of 15 krads of mixed gamma-neutron radiation. Following irradiation, the animals were intravenously injected with horseradish peroxidase (HRP) and sacrificed. The medullae of these animals were fixed either by perfusion or by immersion, incubated in a substrate medium and processed for electron microscopic examination. In control animals subjected to the same procedures, the reaction product of the enzyme HRP and its substrate was confined to the lumina of the blood vessels in the brain fixed by immersion and completely washed out of the blood vessels of the brain fixed by perfusion. The brain tissue of the irradiated animals injected with HRP and fixed by immersion exhibited a dense reaction product in the lumina of the blood vessels, in invaginations of the luminal surface, and in micropinocytotic vesicles of the endothelial cell cytoplasm. The electron dense material was also observed in the outer margin of the basement membrane and in areas beyond this membrane. The brain tissue of the irradiated animals fixed by perfusion was similar to the tissue fixed by immersion except for the presence of reaction products within blood vessels. The most significant alteration in the medullary tissue of the irradiated animals was an increase in the number of micropinocytotic vesicles in the endothelial cells. This was viewed as the mode of transcapillary passage of substances from the vascular tissue to the parenchymal tissue of the brain. It appears, then, that damage caused by high doses of radiation initiates increased vascular permeability which may lead to the edematous condition observed in the irradiated animal.

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